Nrf2 role in immune surveillance

Sara Mikac [1], Alicja Sznarkowska [1#], Robin Fahraeus [1,2,3,4#]

[1] *International Centre for Cancer Vaccine Science University of Gdansk Wita Stwosza 63, 80-308 Gdansk.* [2] Université Paris 7, Inserm, UMR 1162, Paris, France.
[3] Department of Medical Biosciences, Umeå University, Umeå, Sweden. [4] RECAMO, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech Republic.

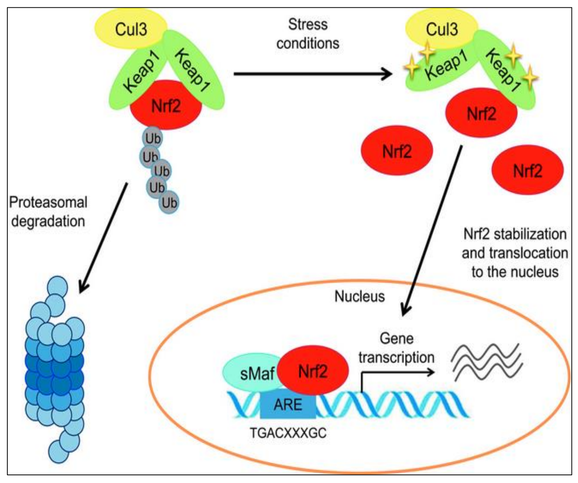
[#] alicja.sznarkowska@ug.edu.pl, robin.fahraeus@inserm.fr

Objectives

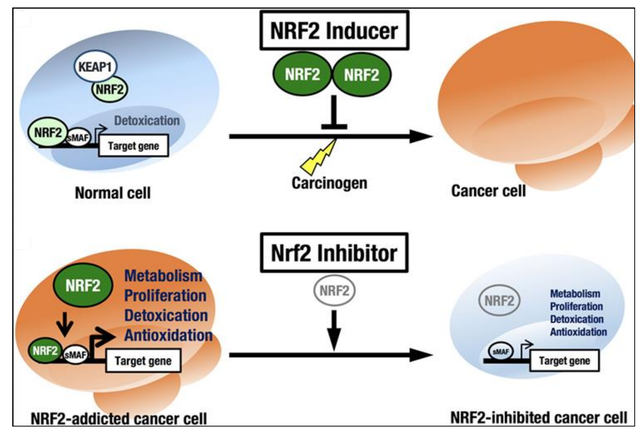
The nuclear factor erythroid 2-related factor 2 (Nrf2) is a relevant basic leucine zipper (bZIP) transcription factor that is essential in the regulation of cell cycle homeostasis, cytoprotection, and innate immunity when cells are under stressful conditions. Nrf2 was shown to protect cells against multiple redox-induced and xenobiotic-induced diseases including cancer and its activation is beneficial in terms of prevention of chronic diseases (Jaramillo and Zhang, 2013). Keap1 is the molecular switch that controls activation and inactivation of the Nrf2 pathway. In response to oxidative or electrophilic stress, Keap1-dependent ubiquitin ligase activity is inhibited and the Nrf2 protein is accumulated. Subsequently, Nrf2 translocates to the nucleus to activate transcription of the ARE-containing genes (Tonelli et al., 2018).

Though Nrf2 function in mounting an immune response is still unsolved, it is widely accepted that Nrf2 speeds up growth and proliferation of cancer cells and confers chemoresistance (Lau et al., 2008).

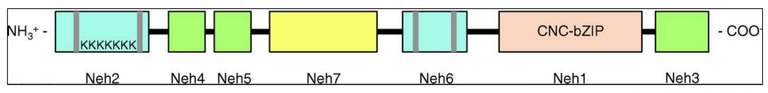
Here we show that **depletion of Nrf2 decreased MHC class I protein and cell surface level, in normal lung fibroblasts and in non-small cell lung cancer cell line (A549) with functional knockout of Nrf2.** Interestingly, this effect was not observed on transcriptional level where the depletion of Nrf2 increased the expression of MHC class I, in both normal lung fibroblasts and A549 cells. It leads to the assumption that **Nrf2 can regulate translation of MHC class I molecules, or affect the degradation of HLAs.**



Tonelli et al., 2018



Taguchi et. al, 2017



Tonelli et al., 2018

Methods

1. Nrf2 knockdown
Small-interfering RNA (siRNA, Santa Cruz Biotechnology) in concentration of 25 nM, with 5µl/well of Lipofectamine 3000 reagent (Invitrogen, Thermo Fisher Scientific) was delivered to normal lung fibroblasts (NLF, RIEKEN). Cells were treated for 48h (qPCR and western blot) or 72h (FACS).

2. Functional Nrf2 KO cells
CRISPR/Cas9-induced NRF2 knockout in A549 cells (clone 2-11) was kindly provided by Prof. Eric Kmiec (Gene Editing Institute, Christiana Care Health System, Newark, United States). The CRISPR-directed gene-editing system was designed to disable the transactivation Neh5 domain of Nrf2 bearing nuclear export signal (NES), which reduces the capacity of the protein to re-enter the nucleus. Steady-state level of MHC class I molecules in wt A549 cells (NSCLC cell line, adenocarcinoma, RIEKEN) and A549 Nrf2 KO cells was measured with qPCR, western blot and FACS.

Discussion

RNA levels of HLA-A and β2-microglobulin are upregulated after Nrf2 depletion, but at the protein and cell surface MHCI level, the opposite effect was observed. Nrf2 knockdown drastically reduced HLA-A and HLA-C molecules, as shown in western blot and decreased the MHCI molecules on cell surface.

Keap1 depletion, despite resulting in Nrf2 accumulation, does not significantly increase cell surface HLAs. The reason for that might be that there are more Keap1 interacting proteins that can affect MHCI levels independent from Nrf2.

Comparing wt A549 cells and A549 cells with functional knockout of Nrf2, the RNA levels of HLA-A, HLA-B and β2-microglobulin were significantly higher after Nrf2. Interestingly, western blot analysis showed opposite results. Steady-state levels of HLA-A and HLA-C were reduced after Nrf2 KO. These observations were also confirmed with flow cytometry, where the steady-state expression of MHCI on the cell surface was significantly lower in A549 KO cells than in wt cells.

Surprisingly, Nrf2 transcriptional target NQO1 did not show significant difference in expression, comparing to wild-type (wt) cells while the reduction on the protein level was massive. It is possible that cells, in the condition of Nrf2 knockout, activate other pathway that can regulate NQO1 expression, which suggests that NQO1 cannot be only indicator of Nrf2 transcriptional activity.

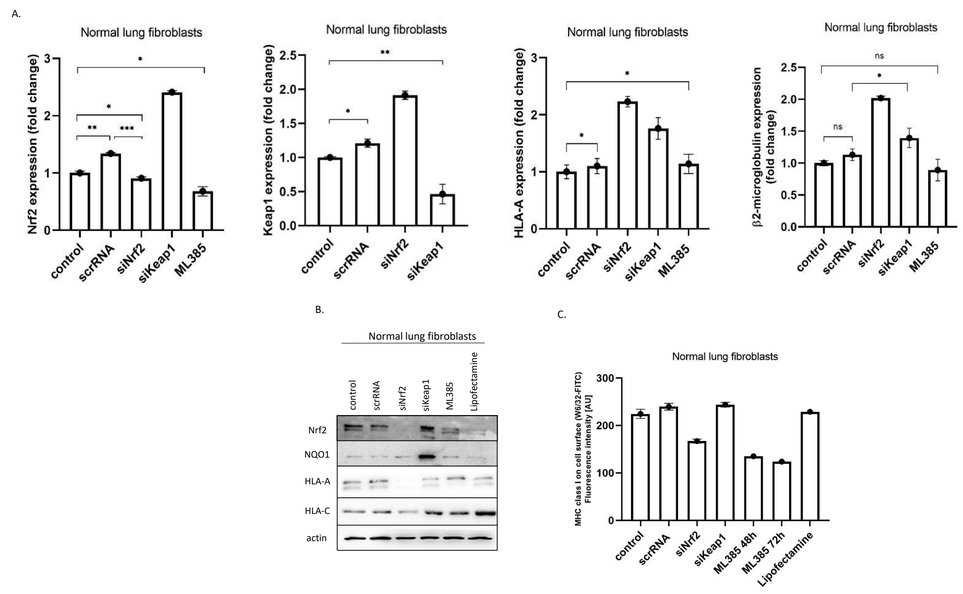
Conclusions

1. Depletion of Nrf2 decreased MHC class I protein and cell surface levels, both in normal lung fibroblasts and in A549 cells with functional knockout of Nrf2, which leads to the assumption that indeed Nrf2 has a role in **immune surveillance**, and by inducing MHC class I, it can induce immune response and protect cells from stress stimuli.

2. Since the opposite effect was observed on transcriptional level, where depletion of Nrf2 increased the MHC class I RNA levels, in both normal lung fibroblasts and A549 lung adenocarcinoma cell, it leads to the assumption that Nrf2 can regulate **translation** of MHC class I molecules. Alternatively, Nrf2 can also affect **degradation** of HLAs.

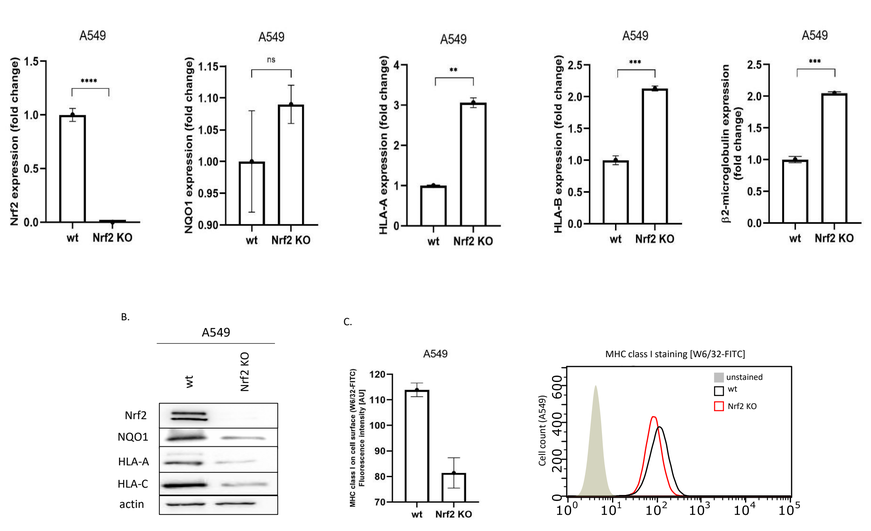
Results

1. Nrf2 depletion in normal lung fibroblasts reduced MHC class I protein and cell surface levels



A. qPCR results from Nrf2 and Keap1 knockdown in normal lung fibroblasts, 48 hours after transfection. **MHC class I RNA levels are increased upon Nrf2 depletion.**
B. Western blot analysis from Nrf2 and Keap1 knockdown in normal lung fibroblasts, 48 hours after transfection. **MHC class I protein levels are decreased upon Nrf2 depletion.**
C. FACS analysis from Nrf2 and Keap1 knockdown in normal lung fibroblasts, 72 hours after transfection. Cell pellets were stained with marker for MHCI expression on cell surface (W6/32-FITC antibodies). **MHC class I cell surface levels are decreased upon Nrf2 depletion.**

2. Functional knockout of Nrf2 reduced MHC class I expression on protein and cell surface levels, but not on RNA level



A. qPCR results of A549 wild type cells and A549 cells with functional knockout of Nrf2, 48 hours after transfection. **MHC class I RNA levels are higher in Nrf2 KO cells comparing to wt.**
B. Western blot analysis, of A549 wild type cells and A549 cells with functional knockout of Nrf2, 48 hours after transfection. **MHC class I protein levels are reduced in Nrf2 KO cells.**
C. FACS analysis of A549 wild type cells and A549 cells with functional knockout of Nrf2, 72 hours after transfection. Cell pellets were stained with marker for MHCI expression on cell surface (W6/32-FITC antibodies). **MHC class I cell surface levels are reduced in Nrf2 KO cells.**

NATIONAL SCIENCE CENTRE POLAND
European Funds Smart Growth
FNP Foundation for Polish Science
European Union European Regional Development Fund